Reproductive cycle of *Scrobicularia plana* (da Costa, 1778) (Bivalvia: Semelidae) in two Moroccan lagoons: Khnifiss and Oualidia

Ciclo reproductor de *Scrobicularia plana* (da Costa, 1778) (Bivalvia: Semelidae) en dos lagunas marroquíes: Khnifiss y Oualidia

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ABSTRACT

The gametogenic cycle of *Scrobicularia plana* (da Costa, 1778) (Mollusca: Bivalvia) was studied in two Moroccan lagoons, both of them exposed to upwelling influence but with different climate and environmental characteristics (Oualidia located in Centre near an industrial area, and Khnifiss in saharian zone). The gonadal development was studied in both populations by histology and gonadic index. At both lagoons, the sex ratio averaged 2:1 in favor of females (at Oualidia, χ^2= 36.4150; df= 24; p= 0.05, and at Khnifiss, χ^2= 31.4104; df= 20; p= 0.05). The gametogenesis cycle began at Oualidia in September, while at Khnifiss in October. Two periods of spawning in spring and summer months were observed in both sites with small differences. Gonadic index never reached the value 1, and in the gonad of all individuals different stages of gametogenesis were found.

RESUMEN

El ciclo gametogénico de *Scrobicularia plana* (da Costa, 1778) (Mollusca: Bivalvia) fue estudiado en dos lagunas de Marruecos, ambas expuestas a la influencia de upwelling, pero con distintos climas y características ambientales (Oualidia situada en la parte mediana de la costa cerca de una zona industrial y Khnifiss en la zona sahariana). El desarrollo de las gónadas se ha estudiado en las dos poblaciones por histología y por el índice gonadal. En ambas lagunas, la proporción de sexos era en promedio 2:1 a favor de las hembras (en Oualidia, χ^2= 36,4150, df= 24, p= 0,05 y en Khnifiss, χ^2= 31,4104, df= 20, p= 0,05). El ciclo de gametogénesis comenzó en septiembre en Oualidia y en octubre en Khnifiss. Dos períodos de puesta en los meses de primavera y verano se observaron en ambos sitios con pequeñas diferencias. El índice gonádico nunca ha alcanzado el valor 1, y en la gónada de todos los individuos se han encontrado distintas etapas de la gametogénesis.

INTRODUCTION

The lagoons are part of coastal environments, and are among the most productive natural marine ecosystems but remain the weakest and most vulnerable because of natural and human effects. In Morocco, there are four lagoons, with

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different bio-ecological and climatic characteristics: Nador lagoon (on the Mediterranean coast) and Moulay Bousselham, Oualidia, and Khnifiss (located on the Atlantic coast). They have important ecological, biological and socio-cultural values, and are most important wetlands as stopover and wintering sites for thousands of migratory birds (Lakhdar Idrissi, Orbi, Zidane, Hilmi, Sarf, Massik and Makaoui, 2004).

The few studies realized on the Moroccan lagoons, have been limited in time and to some aspects: Chemical contamination (Cheggour, Chafik, Langston, Burt, Benbrahim and Texier, 2001), avifauna (Radi, Bergier, El Idrissi, Qninba, Zadane and Dakki, 2009; Zadane, Qninba, Ibn Tattou and Bergier, 2009), hydrology (Lakhdar Idrissi *et al.*, 2004).

In the last years, a multidisciplinary program (physical chemistry, structure of macrobenthic communities, pollutants, ecotoxicology and microbiology) has been carried out by our laboratory in two lagoons: Khnifiss and Oualidia (Idaradare, Chiffoleau, Moukrim, Alla, Auger, Lefrere and Rozuel, 2004). As a part of this research program, we have studied the reproductive cycle of : *Scrobicularia plana* (da Costa, 1778) an important and characteristic species of lagoon ecosystems.

Scrobicularia plana is a common intertidal sentinel species inhabiting soft substrata in estuaries and coastal areas from the Atlantic and the Mediterranean, with a wide range of distribution (Tebble, 1976). Because of this many studies have involved it (Paes-Da-Franca, 1956; Hughes, 1971; Worrall, Widdows and Lowe, 1983; Zwarts, 1991; Essink, Beukema, Coosen, Craeymeersch, Ducrotoy, Michaelis and Robineau, 1991; Sola, 1997; Guerreiro, 1998; Rodríguez de la Rúa, Prad, Romero and Bruzón, 2003; Raleigh and Keegan, 2006).

In Morocco, studies on the biology of *Scrobicularia plana* have concerned the dynamics of population monitoring (Kourradi, 1987; Cheggour, 1988; Bazairi, 1999). The reproductive cycle of *S. plana* was studied at the estuary of Oued Souss (Agadir) through histological studies (Bergayou, 2006).

In the present work, we study the reproductive biology of this species by histological techniques from two lagoons, Oualidia (northern industrial area) and Khnifiss (saharan zone), different by their climate and environmental characteristics, but both of them exposed to the influence of upwellings.

MATERIALS AND METHODS

Study sites (Fig. 1)

Oualidia lagoon (32° 44′ 42″ N, 9° 02′ 50″ W) is located between El Jadida and Safi in a highly industrialized urban area on the Atlantic coast. This lagoon is 7 km long and on average 0,5 km wide, and exchanges water with the ocean through a major inlet about 150 m wide and 2 m deep. Rainfall over the region accounts only for 1% of the fresh water entering the lagoon. The annual average rainfall, estimated from 1977 to 1998, is about 390 mm, with a maximum in December and no rain in the dry period from autumn (October) until spring. The drought began in June and continued until September. There are two large industrial complexes around this coast: Jorf Lasfar and Safi. Banaoui, Chiffoleau, Moukrim, Burgeot, Kaaya, Auger and Rozuel (2004) have reported a high contamination of Cd (29 μg/g dw) in *Perna perna* near the main discharges from these industrial complexes. Similar results were reported by Chafik, Cheggour, Cossa and Sifeddine (2001) who studied the contamination of *Mytilus galloprovincialis* in the same sites.

The Khnifiss lagoon (20 km long and 65 km2 surface area) is located between Tantan and Tarfaya (28° 02′ 54″ N, 12° 13′ 66″ W). It opens up into the Atlantic Ocean through a narrow inlet called "Foum Agoutrir", about 100 m wide. This lagoon, far from anthropogenic activity is part of the national biological and ecological reserve created on September 2006 (Parc National de Khnifiss, décret n° 2.06.461 of 26 September 2006).The lagoon is the only one located in the Saharian bioclimatic level. The water circulation to the lagoon is rich in nitrogen and phosphate components, especially during spring

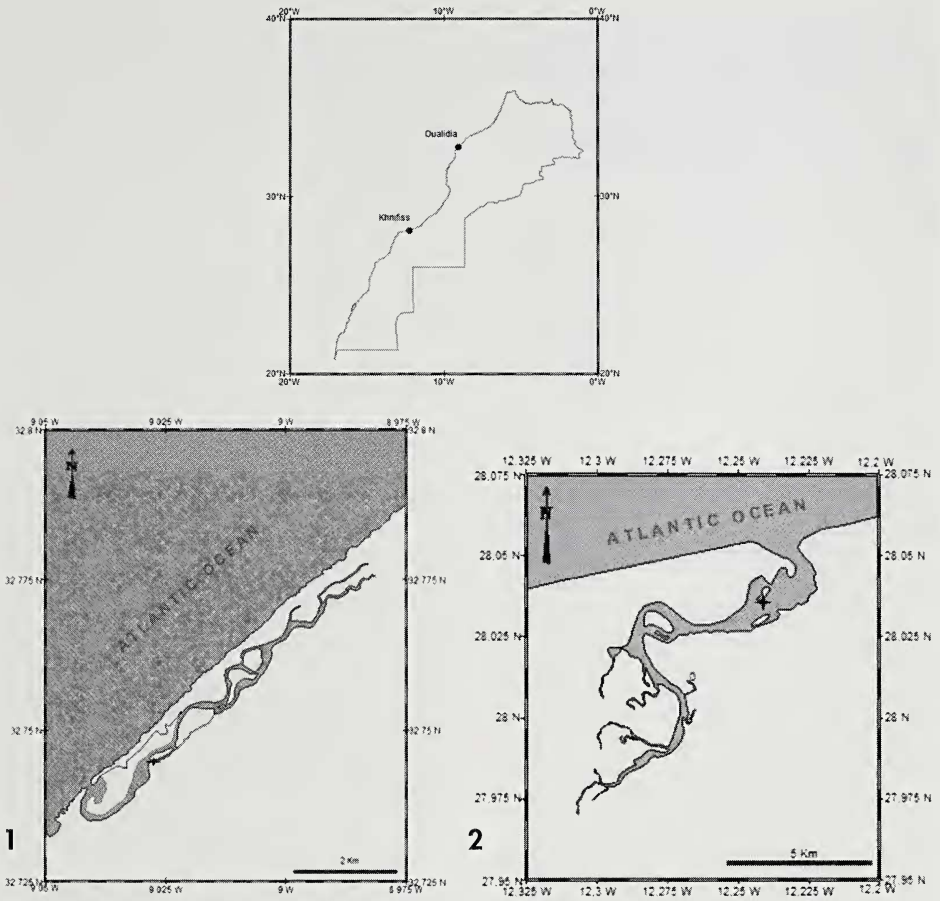

Figure 1: Location of the lagoons of Oualidia (1) and Khnifiss (2).
Figura 1: Situación de las lagunas de Oualidia (1) y Khnifiss (2).

tides. The high amount of chlorophyll in the lagoon is related to the presence of upwelling in the coastal area during spring and summer months. Previous studies have shown that the lagoon of Khnifiss is an environment recommended for shellfish (LAKHDAR IDRISSI *ET AL.*, 2004).

Sampling

Samples of *Scrobicularia plana* were collected from January 2005 to January 2006 in Oualidia, and Khnifiss, except January and September for the latter lagoon. Samples were taken in mud flats on a monthly frequence. Water temperature was recorded in each sampling period.

Reproductive cycle parameters studied

In total 905 specimens were sampled, 582 from Oualidia and 323 from Khnifiss (shell length 23 to 32 mm). For histological study of gonads, shells of *S. plana* were opened and soft parts were fixed in Bouin for 24 h. In the laboratory, the shell of each bivalve was removed and small pieces of soft parts were post-fixed in a new solution of Bouin. The central region of the foot was removed before being dehydrated through a graded series of ethanol and butanol, and finally embedded in wax. Sections 7 μm in thickness were then cut and stained with Hematoxilin of

Table I. Gametogenic scale used to determine histological stage of the gonadic developmental in *S. plana* according to LUBET (1959).
Tabla I. Escala gametogénica utilizada para determinar el estadio histológico de desarrollo de la gónada en S. plana, *según LUBET (1959).*

Stage	Brief description of gonad
Stage 0	Sexual rest
Stage I	Early gametogenesis
Stage IIID	Spent, completely empty lumina
Stage II	Actively developing gonads but mature gametes were not observed
Stage IIIA	Near ripe follicles with mature gametes
Stage IIIB	Spawning follicles distended
Stage IIIC	Partial spawning, partially empty lumina

Carazzii/ eosin (MARTOJA ET MARTOJA, 1967). The different stages of gonadal development were scored according to LUBET (1959) (Table I).

Quantitative analysis of the reproductive cycle was estimated from the gonadic index. This index (Seed, 1975) indicates the state of gonad maturity for each population and it is obtained from histological data. It was determined by giving a number to each of the gametogenic stages of LUBET (1959): stage 0 (number 1), stages I and II (number 2), stage IIIA (number 3), stages IIIB and IIIC (number 2) and stage IIID (number 1). For each sample of clams, the number of gonads showing a gametogenic stage is multiplied by the corresponding number. The figures obtained were then added and the sum was then divided by the total number of clams studied. This index broadly defines the reproductive condition of the population at any particular time. An increasing index in successive samples indicates that gonads are developing, while a falling index means that spawning is in progress.

RESULTS

Sex ratio

During this study, approximately 6.18% and 7.43% of the sampled population, at respectively Oualidia and Khnifiss, could not be sexed. The sex ratio was determined during the months of sexual activity, when the sexes are easily recognizable. In Oualidia, 546 specimens were analysed; the percentage of females and males was respectively 63 and 37 %. The sex ratio averaged 2:1 in favour of females (χ^2= 36.4150; df= 24; P= 0.05). At Khnifiss, a total of 299 individuals were analysed. The males represented 37.8 % and females 62.2 %. The sex ratio is in favour of females 2:1 (χ^2= 31.4104; df= 20; P= 0.05).

Gametogenic cycle

The distribution of the gametogenesis stages in *Scrobicularia plana* (both females and males) of Oualidia or Khnifiss lagoon, according to LUBET's scale (1959) is given in Figures 2 and 3. The gametogenic cycle of the populations from both sites shows few differences.

In clams from Oualidia lagoon, the stage 0 (sexual rest) and 1 (early gametogenesis) seems to begin from September (20% S0, 25% S1) to December (39.13% S1) in females. Developing gonads with mature gametes, stages II (16.67% in January) and IIIA (41.67% in May) occurred from January to August with peaks of spawning in both females and males. In this period, males from Oualidia were either ripe (IIIA stage) or in spawning (IIIB stage). From April to August, females were also either ripe or spawning. In males, we observed IIIC stage (17.64%) from April and IIID (33%) stage from May.

The population of Khinifiss showed stages 0 and 1 from October to December (as revealed in males). Some individuals in development of gonad were also found in this period. Stage IIIA (15%) was observed in early October and continued with important percentages of females until March (23%).Peaks of spawning (IIIB stage) occurred in August and November-December. The spawning of August was the most important in both females and males (50%). Increasing percentages of Stages IIIC and IIID have been observed from early April.

Gonadic index

In Oualidia lagoon, gonadic index shows increasing values from February to April (stage I and II). Principal spawning occurred in March which seems to be related to an increase in temperature levels. From April, there was a decrease in gonadic indices (stage IIIB) together with a decrease of sea water temperature. After spawning, a reconstruction of the gonad and additional spawning were observed, (optional periods of spawning were observed in September and November) (Figure 4).

In Khnifiss lagoon, gonadic index decreases between February and April, which corresponded to a spawning period and was coincident with a decrease of the temperature values. In July, when temperature levels increase, a further spawning event was recorded. The rest of the active period corresponds to reconstructions of the gonad and additional spawning.

In both sites, the gonadic index never reached 1. This shows that the resting period is reduced in *Scrobicularia plana* in Oualidia and Khnifiss lagoons.

Temperature

Figure 4 shows the variations of temperature along the sampling period. A similar temporal pattern was observed in both lagoons. The lowest values (15 °C) were observed in December whereas for the warm period (April-October), values were between 20 and 30°C. In Oualidia lagoon (Fig 4A), the temperature shows increasing levels from January to April. Another peak has been detected in October.

In Khnifiss lagoon, the temperature increases from February to April followed by a small decrease until July. Maximum value was also observed in August.

DISCUSSION

Over the course of our investigations the reproductive cycle of *Scrobicularia plana* was studied in Oualidia and Khnifiss lagoons. We have used specimens with shell length ranging between 20 and 32 mm (both sites considered). RALEIGH AND KEEGAN (2006) had reported that sexual maturity was attained at approximately 22 mm in shell length in Mweelon Bay (Galway, west coast of Ireland). A similar value was also reported by PAES-DA-FRANCA (1956), SOLA (1997) and GUERREIRO (1998). HUGHES (1971) observed that *S. plana* reaches sexual maturity in the second summer of life, in the animals with a shell length of 20 mm. RODRÍGUEZ DE LA RÚA *ET AL.* (2003) reported that the smallest size analyzed measured 23 mm in the Guadalquivir estuary (South West of Spain).

No hermaphroditic animals were observed during our study. HUGHES (1971), SOLA (1994) and RODRÍGUEZ DE LA RÚA (2003) obtained the same results, and established that *S. plana* is predominantly a dioecious species. However, PAES-DA-FRANCA (1956) reported a maximum of 4.42% showing this condition in a Portuguese population, and RALEIGH AND KEEGAN (2006) indicate one hermaphroditic condition in a Mweelon Bay (Galway, west coast of Ireland) population. Although this condition is rare in *S. plana*, LAMMENS (1967) indicates that it is usual to find a small number of hermaphrodite specimens in gonochoristic bivalve species, like in the case of *Anodonta cygnea* (Linné), *Mytilus edulis* (Linnaeus) and *Dreissena polymorpha* (Pallas).

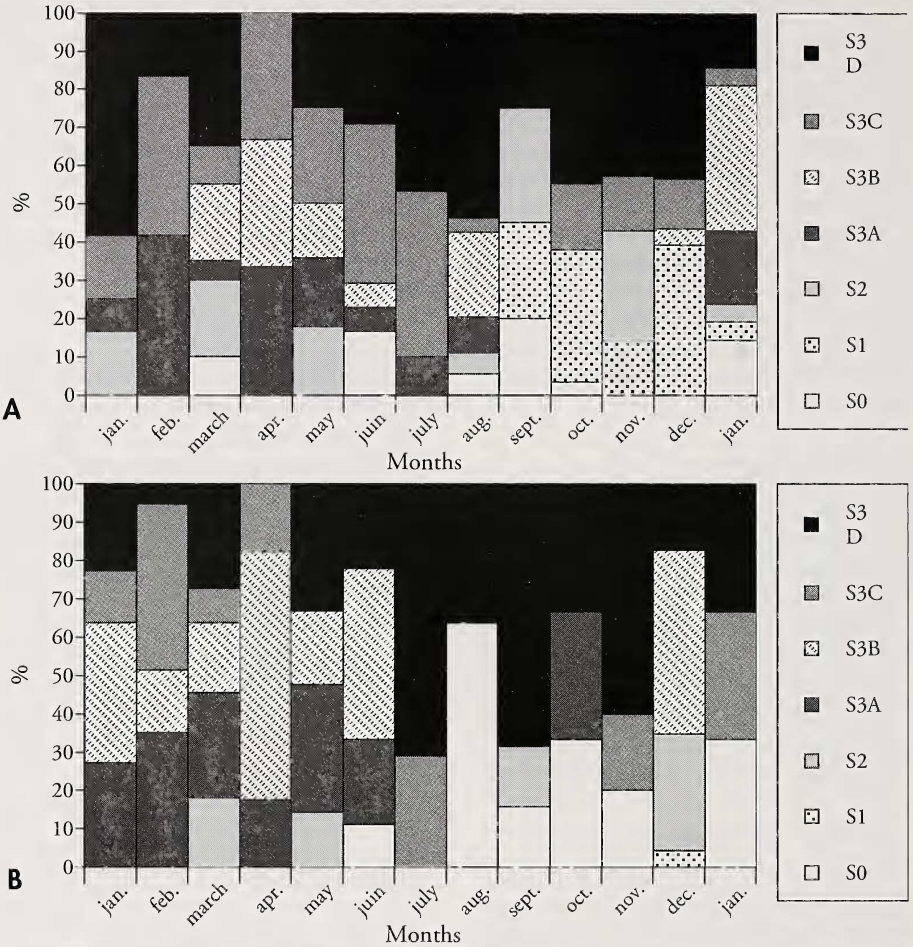

Figure 2. Percentage of occurrence of different stages of gametogenesis in *Scrobicularia plana* sampled from Oualidia lagoon (A: females; B: males). The cumulated frequencies of the different developmental stages corresponded to all bivalves studied (100%).

Figura 2. Porcentaje de ocurrencia de las diferentes etapas de la gametogénesis en Scrobicularia plana *muestreada en la laguna de Oualidia (A: hembras, B: machos). Las frecuencias acumuladas de las distintas etapas de desarrollo corresponden al total de los bivalvos estudiados (100%).*

At both lagoons, the overall sex ratio recorded by the current study indicates a predominance of females over males (at Oualidia: 63 % to 37 %, χ^2= 36.4150; df= 24; P= 0.05; at Khnifiss: 62.2 % to 37.8 %, χ^2= 31.4104; df= 20; P= 0.05). Otherwise, in other studies, Paes-da-Franca (1956) had also found a slightly higher number of females than males (1.2:1). By the same token, RALEIGH AND KEEGAN (2006) reported a rate of 1.1:1, but balanced it to 1:1.

In this study, we observed two principal periods of spawning in populations, the first in spring and the second in summer. In Oualidia lagoon, gonad development in *Scrobicularia plana* began in September (early autumn), and the spawning period began in March (spring). *S. plana* from this lagoon showed an extended period of gamete release, from March to August. Additional spawning periods have been recorded along the study period (espe-

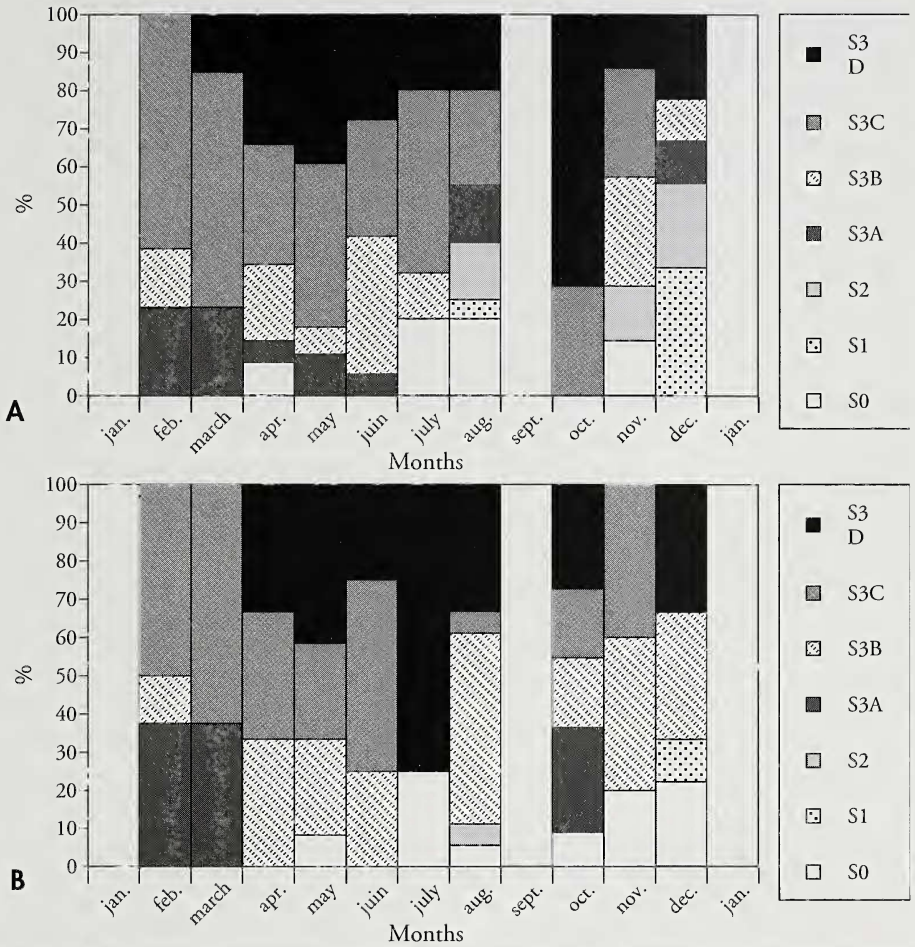

Figure 3. Percentage of occurrence of different stages of gametogenesis in *Scrobicularia plana* sampled from Khnifiss lagoon (A: females; B: males). The cumulated frequencies of the different developmental stages correspond to all bivalves studied (100%).

Figura 3. Porcentaje de ocurrencia de las diferentes etapas de la gametogénesis en Scrobicularia plana *muestreada en la laguna de Khnifiss laguna (A: hembras, B: machos). Las frecuencias acumuladas de las etapas de desarrollo diferentes corresponden al total de los bivalvos estudiados (100%).*

cially in September (late summer) and in November (autumn). In Khnifiss, the gonad development began in October and the spawning occurred in April and July (it seems that this is caused by an increase of temperature values). Active gametogenesis continued with reconstruction of gonad and additional spawning periods. Gonadic index never reached the value of 1, and all individuals were at various stages of gametogenesis evolution.

According to HUGHES (1971) and ESSINK *ET AL.*, (1991), the differences in the prolongation of the reproductive cycle of *S. plana*, would be due to latitudinal and thermal differences along the Atlantic coast (SOLA, 1997). At first, the start of gonad development is reached when mean temperature was an average of 20°C (Oualidia: 15°C to 25°C; Khnifiss: average of 20°C). In Spain, the start of gametogenesis coincided with an increase of temperature in the

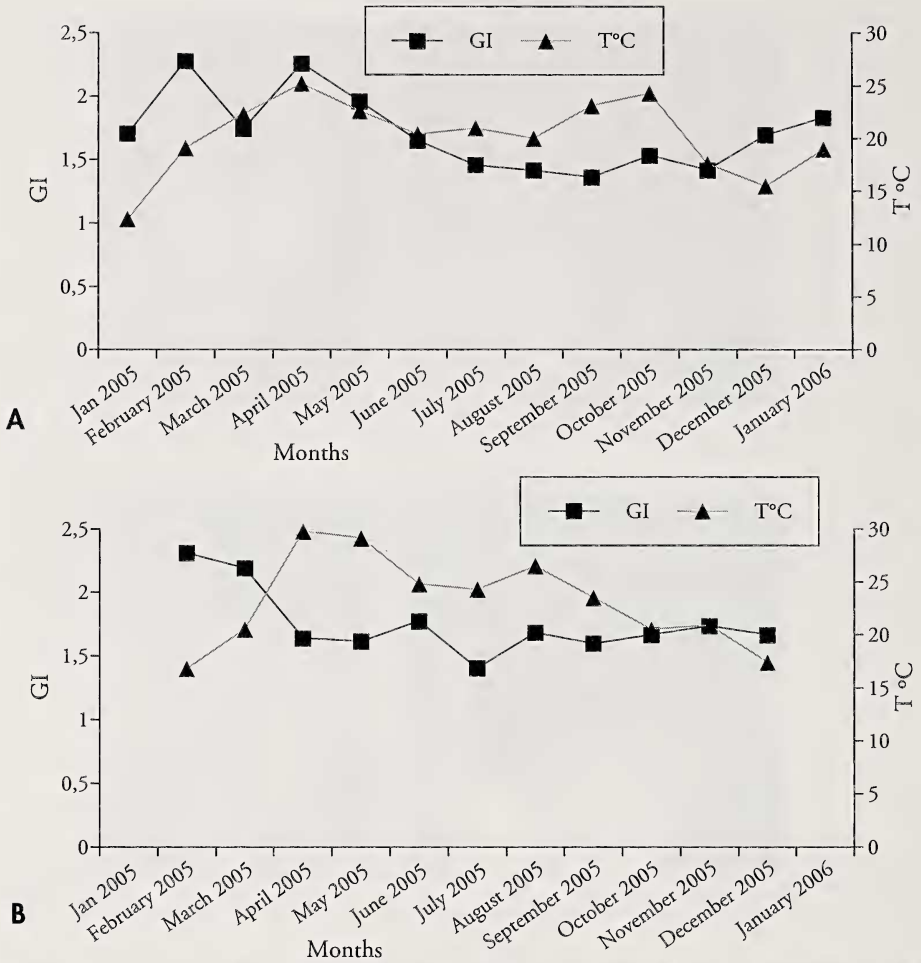

Figure 4. Variation of gonadic index (squares) and water temperature (triangles) throughout the study period. A: Oualidia; B: Khnifiss.

Figura 4. Variación del índice gonádico (cuadrados) y de la temperatura del agua (triángulos) durante el período de estudio. A: Oualidia; B: Khnifiss.

months of January (Sola, 1997) and February (Rodríguez de la Rua *et al.*, 2003). Hughes (1971), in his study in North Wales, found development of gonad to begin at a later date (April). Raleigh and Keegan (2006) found that gonad development began in January/February when the water and substrate temperature were, on average, 9°C.

Paes-da-Franca (1956), in a population of *Scrobicularia plana* of Portugal, found differentiated gametes in nearly all months of the year, except a short resting period in December with temperature of ca. 14 °C. The same result was reported throughout the current study, and we found differentiated gametes in all months of the sample period (except during the resting period). An increase of temperature at Oualidia could be responsible for the beginning of gametogenesis. The time available for gonadal development may decrease in relation to latitude increasing, until just beyond the geographical limit of the species.

According to several authors, *S. plana* is a species whose reproductive cycle varies with latitude (PAES-DA-FRANCA, 1956; HUGHES, 1971; BACHELET, 1981; GUERREIRO, 1998; RODRÍGUEZ DE LA RÚA *ET AL.*, 2003; and RALEIGH AND KEEGAN, 2006) with southern populations exhibiting more than one annual spawning period. Studies conducted in North Wales (HUGHES, 1971), northwest Spain (SOLA, 1997) and the west coast of Ireland (RALEIGH AND KEEGAN, 2006) recorded an increase in development from March onwards, spawning occurring from June to August and gonad regression in September. Studies carried out on Iberian populations (PAES-DA-FRANCA, 1956; GUERREIRO, 1998; RODRÍGUEZ DE LA RÚA *ET AL.*, 2003) reported two spawning periods. In Southern Spain, bivalve spawning periods are known to be longer due to latitudinal characteristics, which is consistent with the February to September spawning period proposed for *Solen marginatus* from Bajo de la Cabezuela (Bay of Cadiz, SW Spain) (BRUZÓN, RODRÍGUEZ DE LA RÚA, ROMERO AND PRADO, 2000): the first one occurring in spring and the second in summer. These results are similar to those found in Moroccan lagoons

WORRALL *ET AL.* (1983), who studied *S. plana* from three different estuaries in Southwest England, observed significant variations between local population, with differences in the timing and duration of the breeding cycle. It is attributed to food availability, suggesting that the shorter time window during which food was available at one site necessitated more rapid gamete development and caused earlier spawning. According to HUGHES (1971) and ESSINK *ET AL.* (1991), the differences in the prolongation of the reproductive cycle of *S. plana* would be due to latitudinal and thermal differences along the Atlantic coast (SOLA, 1997). PAES-DA-FRANCA (1956) and RODRÍGUEZ DE LA RÚA *ET AL.* (2003) observed that variations in the levels of chlorophyll "a" might affect the reproduction cycle of *S. plana*.

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